to the design of new receptor-specific hormones having more useful properties as agonists or antagonists.

EXAMPLE 14

Recruitment of Binding Properties of Human Growth Hormone into Human Placental Lactogen.

Human placental lactogen (hPL) is reduced over thirty-fold in binding affinity compared to hGH for the hGH receptor (G. Baumann, et al., (1986) *J. Clin. Endocrinol. Metab.* 62, 134; A. C. Herington, et al. (1986) *J. Clin. Invest.* 77, 1817). Previous mutagenic studies showed the binding site on hGH for the hGH receptor is located primarily in two regions (including residues 54–74 and 171–185), with some minor determinants near the amino terminus (residues 4–14).

The overall sequence of hPL is 85% identical to hGH. Within the three regions that broadly constitute the receptor binding epitope on hGH, hPL differs at only seven positions and contains the following substitutions: P2Q, I4V, N12H, R16Q, E56D, R64M, and I179M. (In this nomenclature the residue for wild-type hGH is given in single letter code, followed by its position in mature hGH and then the residue found in hPL; a similar nomenclature is used to describe mutants of hGH.) Single alanine substitutions have been produced in hGH at each of these seven positions. Of these, four of the alanine substitutions were found to cause two-fold or greater reductions in binding affinity, including I4A, E56A, R64A, and I179A. Generally, the alanine substitutions have a greater effect on binding than homologous substitutions from human prolactin. Therefore, the effect of some of the substitutions from hPL introduced into hGH were investigated.

Whereas the I179A substitution caused a 2.7-fold reduction in affinity, the I179M substitution caused only a slight 1.7-fold effect. However, the R64A and R64M substitutions caused identical and much larger reductions (about 20-fold) in binding affinity. Moreover, the double mutant (E56D:R64M) in hGH was even further reduced in affinity by a total of 30-fold (Table I). Thus, E56D and R64M primarily determine the differences in receptor binding affinity between hGH and hPL. The double mutant D56E, M64R in hPL therefore substantially enhances its binding affinity for the hGH receptor. Additional modifications such as M179I and V4I also enhance binding of hPL to the hGH receptor.

EXAMPLE 15

Effect of Amino Acid Replacement at Position 174 on Binding to the Human Growth Hormone.

As previously indicated, replacement of Glu174 with Ala(E174A) resulted in more than a 4-fold increase in the affinity of human growth hormone (hGH) for its receptor. To determine the optimal replacement residue at position 174 hGH variants substituted with twelve other residues were made and measured to determine their affinities with the hGH binding protein (Table XXIV). Side-chain size, not charge, is the major factor determining binding affinity. Alanine is the optimal replacement followed by Ser, Gly, Gln, Asn, Glu, His, Lys, Leu, and Tyr.

TABLE XXIV

Mutant[a]	Side chain Charge	Side chain Size(Å³)[b]	Kd (nM)[c]	Kd(mut)/Kd(wild type)
E174G	0	0	0.15	0.43
E174A	0	26	0.075	0.22
E174S	0	33	0.11	0.30
E174D	−	59	NE	—
E174N	0	69	0.26	0.70
E174V	0	76	0.28	0.80
wild-type	−	89	0.37	1.0
E174Q	0	95	0.21	0.60
E174H	0	101	0.43	1.2
E174L	0	102	2.36	6.4
E174K	+	105	1.14	3.1
E174R	+	136	NE	—
E174Y	0	137	2.9	8.6

[a]Mutations were generated by site-directed mutagenesis (Carter, P., et al. (1986) Nucleic Acid Res. 13, 4431–4443) on a variant of the hGH gene that contains a KpnI site at position 178 cloned into pB0475. Oligonucleotides used for mutagenesis had the sequence:

5'-AC—AAG—CTC—NNN—ACA—TTC—CTG—CGC——ATC—GTG—CAG—T-3',

where NNN represents the new codon at position 174 and asterisks indicate the mismatches to eliminate the KpnI site starting at codon 178. Mutant codons were as follows: Gln, CAG; Asn, AAC; Ser, AGC; Lys, AAA; Arg, AGG; His, CAC; Gly, GGG; Val, GTG; Leu, CTG. Following heteroduplex synthesis the plasmid pool was enriched for the mutation by restriction with KpnI to reduce the background of wild-type sequence. All mutant sequences were confirmed by dideoxy sequence analysis (Sanger, F.,et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467).
[b]Side-chain packing values are from C. Chothia (1984) Annu. Rev. Biochem. 53, 537.
[c]Dissociation constants were measured by competitive diplacement of [^{125}I] hGH from the hGH binding protein as previously described. NE indicates that the mutant hormone was expressed at levels too low to be isolated and assayed.

EXAMPLE 16

The hGH variants shown in Table XXV were constructed. Their relativity potency as compared to wt-hGH are shown.

TABLE XXV

hGH mutant	Relative potency in rat weight gain assay
F97A	0.87
S100A	2.12
L101A	3.03
V102A	1.39
Y103A	1.73
T175S	1.21

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a variant of human prolactin, wherein said variant binds to the human growth hormone receptor and has the amino acid sequence of the human prolactin as shown in FIG. 2 except that said variant has 1 to 19 amino acid substitutions compared to said human prolactin. and wherein at least one of the amino acid substitutions is an amino acid substitution at ~~an amino acid~~ residue selected from the group consisting of H54, T55, S56, L58, A59, E62,

VIII. Claims 52-54, drawn to a recombinant protein variant with the ability to modulate an immune response to a naturally occurring allergen, classified in class 514, subclass 12.

IX. Claims 55, 56, 57, 59, drawn to a pharmaceutical composition comprising a protein variant and a pharmaceutically acceptable carrier, classified in class 424, subclass 1.11.

X. Claim 58, 65 drawn to a method of preventing or treating allergy in a patient by administering a pharmaceutical composition, classified in class 424, subclass 1.11.

XI. Claim 60 drawn to a method of generating an immune response by administering a recombinant protein variant, classified in class 514, subclass 12.

XII. Claim 61, drawn to a method of generating an immune response by administering a pharmaceutical composition, classified in class 424, subclass 1.11.

XIII. Claim 62, drawn to a method of vaccination by administering a recombinant protein variant, classified in class 514, subclass 12.

XIV. Claim 63, drawn to a method of vaccination by administering a pharmaceutical composition, classified in class 424, subclass 1.11.

XV. Claim 64, drawn to a method for the treatment of allergic reactions by administering a recombinant protein variant, classified in class 514, subclass 12.

D63, K64, Q66, A67, M70, N71, Q72, K73, D74, H171, N175, Y176, K178, and L179, numbered as shown for said human prolactin in FIG. 2.

2. An expression vector comprising the nucleotide sequence of claim 1.

3.An expression host comprising an expression vector including the nucleotide sequence of claim 1.

4. An isolated nucleic acid comprising a nucleotide sequence encoding a variant of human placental lactogen, wherein said variant binds to the human growth hormone receptor with an affinity different from the affinity of the human placental lactogen for the human growth hormone receptor and has the amino acid sequence of the human placental lactogen as shown in FIG. 2 except that said variant has 1 to 4 amino acid substitutions compared to said human placental lactogen, and wherein at least one of the amino acid substitutions is an amino acid substitution at an amino acid residue selected from the group consisting of Q2, V4, H12, Q16, D56, M64, and M179, numbered as shown for said human placental lactogen in FIG. 2.

5. An expression vector comprising the nucleotide sequence of claim 4.

6. An expression host comprising the expression vector including the nucleotide sequence of claim 4.

7. An isolated variant of human prolactin, wherein said variant binds to the human growth hormone receptor and has the amino acid sequence of the human prolactin shown in FIG. 2 except that said variant has 1 to 19 amino acid substitutions compared to said human prolactin, and wherein at least one of the amino acid substitutions is an amino acid substitution at an amino acid residue selected from the group consisting of H54, T55, S56, L58, A59, E62, D63, K64, Q66, A67, M70, N71, Q72, K73, D74, H171, N175, Y176, K178, and L179, numbered as shown for said human prolactin in FIG. 2.

8. The human prolactin variant of claim 3 wherein said amino acid substitution is selected from the group consisting of H54F, T55S, S56E, L58I, A59P, E62S, D63N, K64R, Q66E, A67T, M70K, N71S, Q72N, K73L, D74E, H171D, N175T, Y176F, and K178R.

9. The human prolactin variant of claim 8 wherein said amino acid substitution comprises H171D, and said human prolactin variant further comprises the following set of amino acid substitutions: N175T: Y176F.

10. The human prolactin variant of claim 9 further comprising the amino acid substitution K178R.

11. The human prolactin variant of claim 10 further comprising the following set of amino acid substitutions: H54F: T55S: S56E: L58I: A59P: E62S: D63N: K64R: Q66E: A67T: M70K: N71S: Q72N: K73L: D74E.

12. The human prolactin variant of claim 10 further comprising the following set of amino acid substitutions:

I184S: H185V: N186E: N187G: N188S and the deletion of the isoleucine between I184 and H185.

13. The human prolactin variant of claim 10 further comprising the following set of amino acid substitutions: H54F: S56E: L58I: E62S: D63N: Q66E.

14. The human prolactin variant of claim 10 further comprising the following set of amino acid substitutions: H54F S56E : L58I.

15. The human prolactin variant of claim 10 further comprising the amino acid substitution D174A.

16. The human prolactin variant of claim 15 further comprising the following set of amino acid substitutions: E62S D63N: Q66E.

17. The human prolactin variant of claim 16 further comprising the amino acid substitution H54F.

18. The human prolactin variant of claim 16 further comprising the amino acid substitution S56E.

19. The human prolactin variant of claim 16 further comprising the amino acid substitution L58I.

20. The human prolactin variant of claim 16 further comprising the amino acid substitution A59P.

21. The human prolactin variant of claim 16 further comprising the amino acid substitution N71S.

22. The human prolactin variant of claim 16 further comprising the amino acid substitution L179I.

23. An isolated variant of human placental lactogen, wherein said variant binds to the human growth hormone receptor with an affinity different from the affinity of the human placental lactogen for the human growth hormone receptor, said variant having the amino acid sequence of the human placental lactogen as shown in FIG. 2 except that said variant has 1 to 4 amino acid substitutions compared to said human palcental lactogen, wherein at least one of the amino acid substitutions is an amino acid substitution at an amino acid residue selected from the group consisting of Q2, V4, H12, Q16, D56, M64, and M1 79, numbered as shown for said human palcental lactogen in FIG. 2.

24. The human placental lactogen variant of claim 23 wherein said amino acid substitution is selected from the group consisting of Q2P, V4I, H 12N, Q16R, D56E, M64R and M179I.

25. The human placental lactogen variant of claim 23 wherein said amino acid substitution is selected from the group consisting of V4A, D56A, M64A and M179A.

26. The human placental lactogen variant of claim 23 wherein said amino acid substitution comprises D56E, and said human placental lactogen variant further comprises the amino acid substitution M64R.

* * * * *

XVI. Claims 67-72, drawn to DNA encoding a recombinant protein, classified in class 536, subclass 23.1.

XVII. Claim 73, drawn to a recombinant protein variant comprising at least one T-cell epitope, classified in class 530, subclass 402.

XVIII. Claim 74, drawn to a diagnostic assay comprising a protein variant, classified in class 435, subclass 7.1.

IXX. Claim 75, drawn to a diagnostic assay comprising a pharmaceutical composition, classified in class 436, subclass 518.

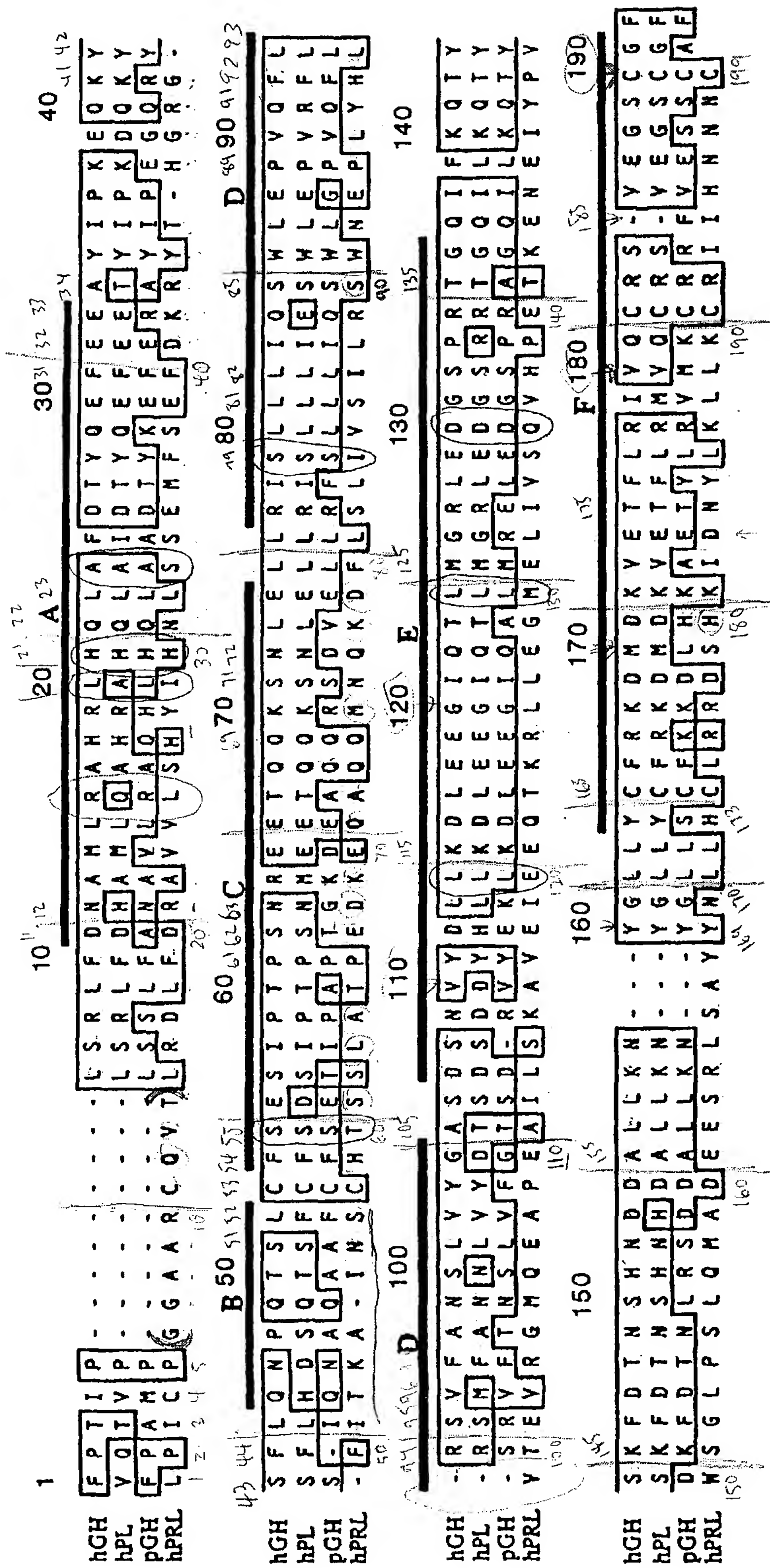

FIG.2

i) 41-57
ii) 94-110
iii) 160-173

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-17, 19, 52-54 drawn to a recombinant protein variant with the ability to induce or modulate a protective immune response to a naturally occurring allergen, classified in class 514, subclass 12.

II. Claims 18, 66, drawn to a method of producing a recombinant protein variant with the ability to induce a protective immune response to a naturally occurring allergen, classified in class 435, subclass 69.1.

III. Claims 20, 33, 34, 35, 36, 37, (38), (39-41), (42-43), (44) drawn to a protein variant where the naturally occurring allergen is an inhalation allergen, classified in class 514, subclass 12.

IV. Claims 21, 22, 23, (24-28), (29-32), drawn to a protein variant where the naturally occurring allergen is a pollen allergen, classified in class 514, subclass 12.

V. Claim 45, 46, drawn to a protein variant where the allergen is an animal allergen, classified in class 514, subclass 12.

VI. Claims 47, 48, 49, 50, drawn to a protein variant where the allergen is a venom allergen, classified in class 514, subclass 12.

VII. Claim 51, drawn to a protein variant where the allergen is a food allergen, classified in class 514, subclass 12.